

Claims

- [c1] 1)A method of concentrating and extracting particles from a blood sample, the method comprising:
exposing the blood sample to an enzyme–detergent combination; and
analyzing the exposed blood sample for the presence of particles.
- [c2] 2)The method of claim 1 wherein the enzyme–detergent combination comprises plasminogen and streptokinase.
- [c3] 3)The method of claim 2 further comprising the steps of:
freezing the plasminogen and streptokinase in coincident relation until a fibrin lysis reagent is needed; and
reacting streptokinase with plasminogen upon thawing whereby plasmin is formed.
- [c4] 4)The method of claim 2 further comprising the step of
suspending the plasminogen in an aqueous salt solution prior to freezing.
- [c5] 5)The method of claim 4 wherein the aqueous salt solution comprises NaCl.
- [c6] 6)The method of claim 4 wherein the aqueous salt solu–

tion comprises NaPO_4 .

- [c7] 7)The method of claim 1 wherein the particles are selected from a group consisting of prions, toxins, metabolic markers, cancerous matter, disease state markers, bacteria, virus, and fungi.
- [c8] 8)The method of claim 1 further comprising the step of replicating the particles through PCR.
- [c9] 9)The method of claim 1 further comprising the step of introducing DNase to the blood sample.
- [c10] 10)The method of claim 1 further comprising the step of introducing Endonuclease to the blood sample.
- [c11] 11)The method of claim 2 wherein the plasminogen and streptokinase are in a dried state.
- [c12] 12)The method of claim 11 wherein the plasminogen and streptokinase are mixed and distributed in disposable test containers.
- [c13] 13)The method of claim 11 wherein the plasminogen is combined with Phospholipase A_2 , DNase, Endonuclease, and Lipase.
- [c14] 14)The method of claim 13 wherein the enzyme-detergent combination is suspended then dried in pellets

of trehalose buffer and packaged as a dry reagent.

[c15] 15)The method of claim 11 wherein the streptokinase is suspended then dried in pellets of trehalose buffer and packaged into tubes as a dry reagent.

[c16] 16)The method of claim 11 further comprising:
resuspending the dried reagents in a buffer;
adding the solution to the volume of blood; and
incubating the sample for at room temperature.

[c17] 17)The method of claim 16, wherein the dried reagent is comprised of 1,500–4,500 KU Phospholipase A₂, 5,000–10,000 U Streptokinase, 2–10 U Plasminogen, 200–3,650 U DNase, 200–4,000 U Endonuclease, and 10,000–100,000 Lipase.

[c18] 18)The method of claim 16 further comprising:
centrifuging the solution;
decanting the supernatant; and
washing the pellet.

[c19] 19)The method of claim 18 wherein the solution is centrifuged for approximately 20 minutes at 5,000–5,500 x g at a temperature of 10–20°C.

[c20] 20)The method of claim 18 wherein the pellet is washed with an Ecotine–HEPES solution.

- [c21] 21)The method of claim 18 wherein the pellet is washed with a Sucrose-HEPES solution.
- [c22] 22)The method of claim 18 wherein the pellet is washed with an Ecotine-HEPES solution and a Sucrose-HEPES solution.
- [c23] 23)The method of claim 16 further comprising:
centrifuging the solution;
decanting the supernatant;
digesting the sample; and
applying the sample to a commercially available nucleic acid extraction method.
- [c24] 24)The method of claim 23 wherein digesting the sample further comprises lysis and DNase inactivation.
- [c25] 25)The method of claim 23 wherein digesting the sample further comprises lysis and Endonuclease inactivation.
- [c26] 26)The method of claim 23 wherein digesting the sample further comprises utilizing proteinase K, sodium dodecyl sulfate, aurintricarboxylic acid, and sodium citrate buffer, incubated at room temperature.
- [c27] 27)The method of claim 16 further comprising:
filtering the solution;
washing the solution;

digesting the sample; and
purifying the extract through commercially available
methods.

[c28] 28)The method of claim 27 wherein digesting the sample
further comprises lysis and DNase inactivation.

[c29] 29)The method of claim 27 wherein digesting the sample
further comprises lysis and Endonuclease inactivation.

[c30] 30)The method of claim 27 wherein digesting the sample
further comprises the steps of:
combining proteinease K, aurintricarboxylic acid, and
sodium citrate buffer;
incubating at room temperature; and
eluting the lysate from the filter surface.

[c31] 31) The method of claim 16 further comprises applying
the solution directly to a biosensor device whereby re-
sponsive to the presence of the pathogens in the blood
sample, the patient develops pathogenic or native dis-
ease state markers which allow for the capture and de-
tection of these markers by the biosensor device.

[c32] 32)The method of claim 16 further comprises applying
the solution directly to a liquid chromatography mass
spectrometry device whereby, responsive to the presence
of the pathogens in the blood sample, the patient devel-

ops pathogenic or native disease state markers that allow for the detection of mass signatures associated with the structural components of the pathogens using the mass spectrometry device.

[c33] 33) The method of claim 16 wherein the buffer comprises Potassium Phosphate, Magnesium Chloride, Sodium Chloride, and Aurintricarboxylic Acid.

[c34] 34) The method of claim 33 wherein the buffer further comprises Triton X-100.

[c35] 35) The method of claim 33 further comprising the step of storing the enzymes with a trehalose buffer.

[c36] 36) The method of claim 35 further comprising the step of combining methyl 6-O-(N-heptylcarbamoyl)- α -D-glucopyranoside and Saponin in the trehalose buffer.

[c37] 37) The method of claim 36 wherein a concentration of 20–35 mM of methyl 6-O-(N-heptylcarbamoyl)- α -D-glucopyranoside is used.

[c38] 38) The method of claim 36 wherein a concentration of 0.05–0.1% Saponin is used.

[c39] 39) The method of claim 35 wherein the trehalose storage buffer comprises Potassium Phosphate, Triton X-

100, Dithiothreitol, and Trehalose.

- [c40] 40) The method of claim 39 wherein the trehalose storage buffer comprises 10 mM Potassium Phosphate.
- [c41] 41) The method of claim 39 wherein the trehalose storage buffer comprises 0.01–0.04% Triton X–100.
- [c42] 42) The method of claim 39 wherein the trehalose storage buffer comprises 1–5 mM Dithiothreitol.
- [c43] 43) The method of claim 39 wherein the trehalose storage buffer comprises 0.3–0.5 M Trehalose.